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Identification of Organic Compounds in a Mutagenic Extract of a Surface Drinking Water by a Computerized Gas Chromatography/Mass Spectrometry System (GC/MS/COM)

(U.S.) Health Effects Research Lab.
Cincinnati, OH

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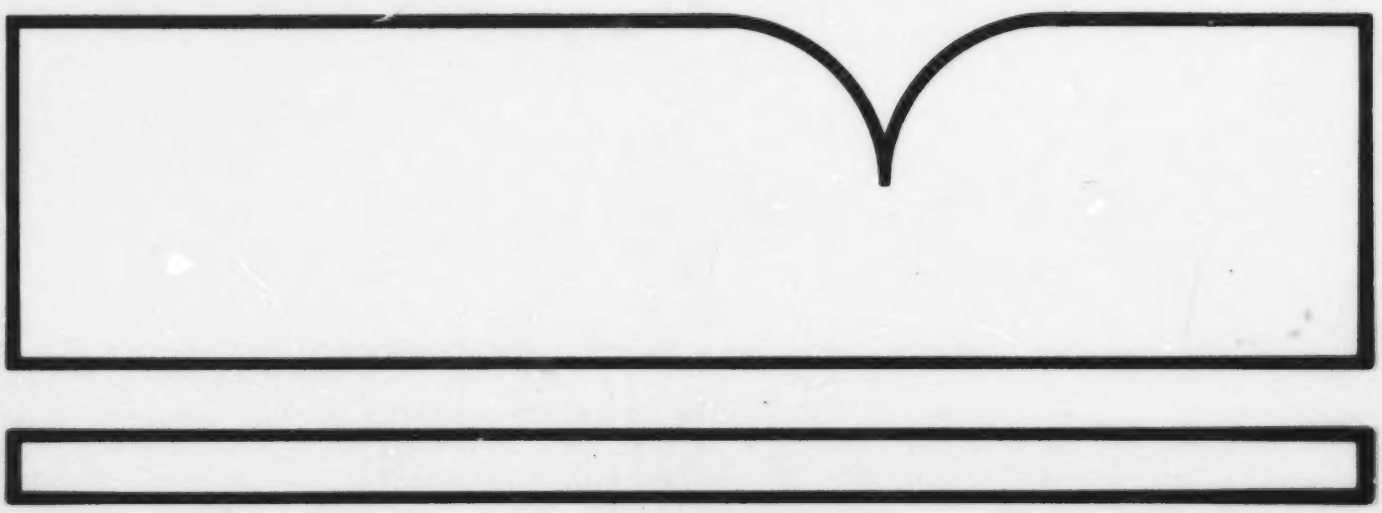

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16. ABSTRACT The organics in a Cincinnati, Ohio drinking water sample were concentrated by a reverse osmosis (RO) process. The diethyl ether soluble extract of the RO concentrate which proved to be mutagenic in studies using the Ames test, was partitioned into acid and base/neutral fractions. The unpartitioned ethyl ether concentrate, an acid and methylated acid fraction, the unpartitioned base/neutral extract, and five base/neutral eluants from a silica gel microcolumn were analyzed for the presence of organics using a computerized gas chromatography/mass spectrometry system (GC/MS/COM) equipped with glass capillary columns. Analysis of individual fractions indicated a predominance of polychlorinated biphenyls (PCBs) and chlorinated aromatics in the second base/neutral partition and many polynuclear aromatics (PNA) in the fourth base/neutral partition. Approximately 460 compounds were identified in this tap water extract, including 41 PNAs, 15 PCBs, and a number of amines, amides, and other halogenated species.

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Identification of Organic Compounds in a Mutagenic Extract of a Surface Drinking Water by a Computerized Gas Chromatography/ Mass Spectrometry System (GC/MS/COM)

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■ The organics in a Cincinnati, Ohio drinking water sample were concentrated by a reverse osmosis (RO) process. The diethyl ether soluble extract of the RO concentrate, which proved to be mutagenic in studies using the Ames test, was partitioned into acid and base/neutral fractions. The unpartitioned ethyl ether concentrate, an acid and methylated acid fraction, the unpartitioned base/neutral extract, and five base/neutral eluants from a silica gel microcolumn were analyzed for the presence of organics using a computerized gas chromatography/mass spectrometry system (GC/MS/COM) equipped with glass capillary columns. Analysis of individual fractions indicated a predominance of polychlorinated biphenyls (PCBs) and chlorinated aromatics in the second base/neutral partition and many polynuclear aromatics (PNA) in the fourth base/neutral partition. Approximately 460 compounds were identified in this tap water extract, including 41 PNAs, 15 PCBs, and a number of amines, amides, and other halogenated species.

The presence of traces of organic chemicals in drinking water has caused concern, since it is thought that they might contribute to the induction of cancer or other illnesses in consumers. The potential health effects of these compounds can be studied only after they are isolated from the water and identified. But since many are of high molecular weight, they cannot be identified by available techniques. Conventional techniques used for isolating organic compounds from water for analytical purposes are not practical for isolating sufficient material for bioassay procedures. Consequently, a technique using reverse osmosis and lyophilization to reduce large amounts of water to volumes that could be extracted in the laboratory while retaining most of the organic carbon was developed (*1*).

In 1974, 400-gal samples of drinking water from the distribution system of Cincinnati, Ohio, were concentrated and extracted by this procedure each month. Eighty percent of the organic matter obtained in each sample was used for biological tests and 20% was retained for chemical analysis if the biological tests were positive. Two of these samples (March and June) were tested for mutagenic activity in the Ames *Salmonella* system (*2*). Both of the concentrates were mutagenic when tested in strains TA100 and TA98 in the presence and absence of an Arochlor-1254-stimulated rat liver homogenate metabolic activation system. Both samples were fractionated by sequential extraction with petroleum ether, diethyl ether, and acetone. When these fractions were assayed, most of the mutagenic activity was in the diethyl ether fraction (*3*).

The sample collected in March was chosen for chemical analysis to attempt to determine the components responsible for the mutagenic activity. The portion of that sample retained for chemical analysis was fractionated in a manner similar to that used to fractionate the bioassay portion. The diethyl ether fraction was then analyzed by glass capillary column gas chromatography/mass spectrometry. This paper reports the results.

Experimental

Sampling and Concentration: Reverse Osmosis Method (RO). The organic pollutants in 400 gal of Cincinnati drinking water, direct from the tap in our laboratory, were concentrated by the RO method of Kopfler (*1*). The theory and practice of RO have been reviewed by others (*4, 5*). Kopfler (*1, 6*), in earlier papers, showed the schematic diagrams of the major components of the dual RO concentrator and of the extraction of the concentrates. The same extraction scheme was used for the subject sample. The pentane and methylene chloride extracts as shown in ref *3* and *6* were combined, dried, and evaporated down to constant weight. The diethyl ether soluble portion of this combined concentrate, which proved to be mutagenic, was the origin of the organic compounds identified in this paper. A detailed procedure for obtaining the ether fraction for GC/MS analysis is described by Melton and Barone (*7*). The rationale for using these methods and the study for which similar samples have been collected have been discussed by Tardiff et al. (*8*).

Fractionation and Partitioning. Burdick and Jackson Distilled in Glass solvents were used for all extractions. The RO concentrate (5–20 mg) was dissolved in 10 mL of ethyl ether and placed in a 10-mL separatory funnel. The organic layer was extracted with four 1-mL aliquots of 5% NaOH and washed with two 1-mL aliquots of distilled water. After separation, the aqueous layer was acidified to pH 2 with 6 M H_2SO_4 solution, saturated with NaCl, and reextracted with four 2-mL aliquots of methylene chloride. The methylene chloride layers were combined and then dried with 0.5 g of sodium sulfate (previously muffled at 400 °C for 1 h). Twenty percent of the dried methylene chloride was set aside for direct GC/MS analysis for acids on glass capillary columns. The remaining solution (80%) was derivatized with diazomethane to form methyl esters from the acids extracted with 5% NaOH (*9*).

The original diethyl ether solution, which contained neutral and basic organics at this point, was reextracted with 1 mL of 5% NaOH, and the aqueous layer was combined with the original aqueous fraction as shown in Figure 1. The ether layer was dried with 0.5 g of sodium sulfate and evaporated in a conical graduated glass tube to 0.5 mL with an Organomation N-Evaporator at 45 °C. (The N-Evaporator allowed one to adjust and maintain a variety of bath temperatures while evaporating the solvent under a stream of nitrogen.) Two milliliters of hexane was added to the tube, and the sample was again evaporated at 60 °C to 0.1 mL. After adding 0.5 mL of hot (60 °C) hexane to the concentration tube, the solution was transferred to the top of a 14 cm × 5 mm i.d. microchromatographic column (disposable transfer pipet) (*10*) packed with 0.2 g of 5% deactivated silica gel (*11, 12*). Organics were partitioned by eluting from the column with the following aliquots of eluants: (1) 0.5 mL of hexane, (2) 1 mL of hexane, (3) 4 mL of hexane, (4) 4 mL of hexane/benzene (1:1), (5) 4 mL of benzene, (6) 4 mL of methylene chloride, and (7) 4 mL of methanol.

Each of the fractions was collected in a premuffled 4-mL graduated glass-stoppered chromaflex sample tube (Kontes, No. K-422560). Eluants 1 to 5 and eluants 6 and 7 were typically evaporated to a volume of 10 to 20 μL at temperatures of 60 and 40 °C, respectively, prior to GC/MS analysis. When the sample volume had to be adjusted, methylene chloride was the solvent of choice. Words of caution: Logsdon (*13*) extracted aqueous solutions containing from 2 up to 900 mg/L residual chloride with methylene chloride and the subsequent GC/MS analysis of the concentrated extracts showed numerous isomers of mono- and dichlorocyclohexanes. If the residual chlorine in the water sample was removed by the addition of sodium sulfite before solvent extraction, then no chlorinated cyclohexanes were observed. The source of these

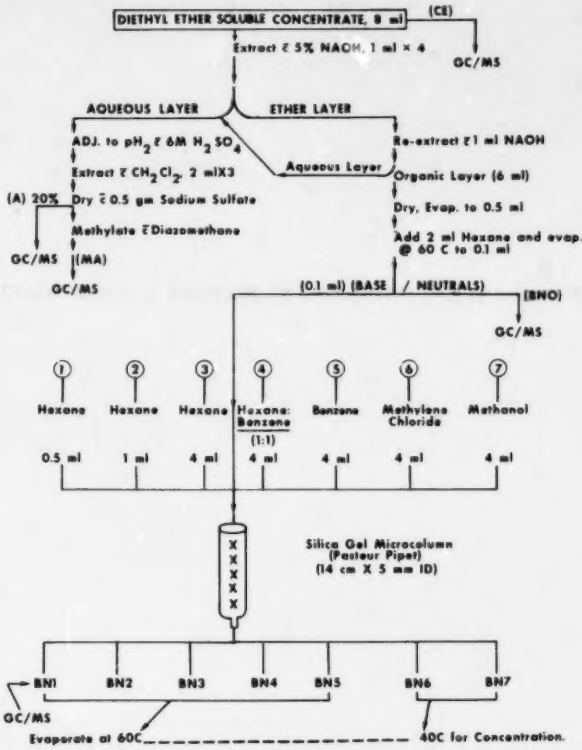

Figure 1. Fractionation, derivatization, and partitioning scheme for the diethyl ether extract

chlorinated organics was traced to the presence of cyclohexene in the methylene chloride. Cyclohexene up to 200 ppm is added by the manufacturer to bulk quantities of methylene chloride as a preservative. The cyclohexene rapidly reacted with the chlorine extracted from the water to form a variety of chlorinated cyclohexanes.

A similar finding was observed in this study even though our concentrates did not contain residual chlorine. However, it is possible that certain concentrations of other reactive chlorine species in the presence of cyclohexene in the methylene chloride or in the water itself could result in similar findings. We were able to cope with this problem by analyzing blanks with the reactive species present. Evidence of this problem can be seen in Figures 2 and 3. Peaks labeled a, b, c, and d are cyclohexane and isomers of chlorocyclohexane, chlorocyclohexanol, and dichlorocyclohexane, respectively. All samples were stored at 0 °C. The detailed schematic of this fractionation and partitioning procedure is presented in Figure 1. Since this study was initiated, the procedure has been refined and is currently being used to fractionate similar RO concentrates (*14*).

Instrumentation. A computerized gas chromatography/mass spectrometry system (GC/MS/COM) was employed to separate and identify organic components of each fraction. The GC/MS/COM included a Finnigan 9500 gas chromatograph and a 3300 mass spectrometer (quadrupole) with a Systems Industries 150 data system.

GC/MS analyses were performed on each fraction using SP1000 (polar) and SE30 (nonpolar) wall-coated open tubular (WCOT) glass capillary columns 30 m long × 0.25 mm i.d. as the separation columns. The columns were interfaced directly to the MS ion source by <0.01-in. i.d. glass-lined stainless steel tubing after deactivating the ends as described by Blomberg (*15*). A Grob designed injector (*16–18*) was installed on the GC for splitless injection of all samples.

For injection of fractions and partitions and their corre-

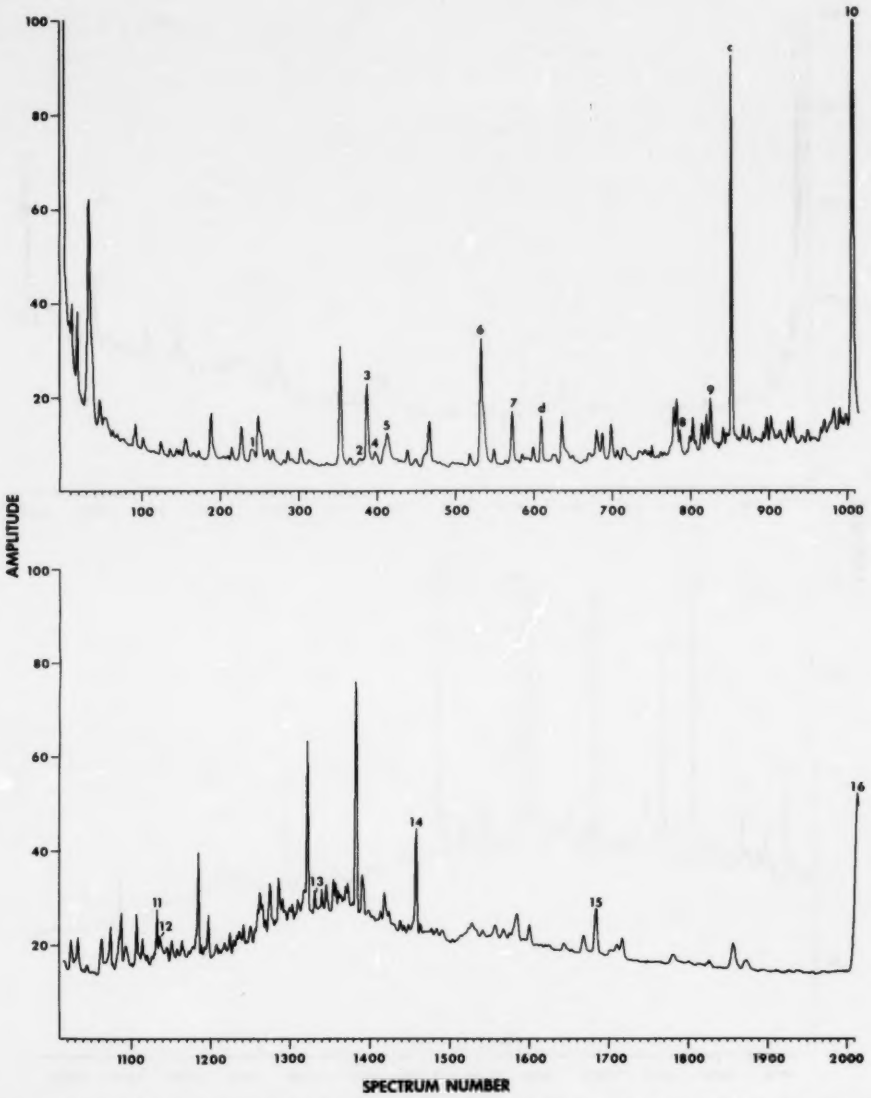

Figure 2. Total ion chromatogram of the unpartitioned diethyl ether extract (CE) chromatographed on SP1000. Numbers identify the following GC peaks: (1) methylpyridine isomer; (2) benzyl chloride; (3) 4-methyl-2-pentanol; (4) ethyl chloroacetate; (5) *N,N*-dimethylformamide; (6) cyclohexanol; (7) cyclohexanone; (8) isophorone; (9) 2-(2-ethoxyethoxy)ethanol; (10) 1-(2-butoxyethoxy)ethanol; (11) benzothiazole; (12) *N*-acetylmorpholine; (13) tetramethylene sulfone; (14) diethyl phthalate; (15) diisobutyl phthalate; (16) dibutyl phthalate

sponding "blanks" on SE30, the injector temperature was maintained at 250 °C. The helium (He) flow through the column was 3 cm^3/min at a linear velocity of 47 cm/s with 40 psi of constant He head pressure after adjusting the Grob splitter to vent at a rate of 57 cm^3/min. (The flow velocity was adjusted by injecting air or pentane at room temperature as an indicator.) The GC oven was temperature programmed from 25 to 270 °C at 2 °C/min. Two microliters (2 μL) of each sample was injected at 25 °C with the Grob vent closed (splitless injection) (*18–21*). The column vent was opened at 30 s and the GC oven temperature program was started as soon as the solvent began to elute from the column into the MS. The same procedure and conditions were used for samples injected on SP1000 with this exception: the column temperature at injection was 50 °C and the GC oven was programmed to 190 °C at 2 °C/min. The MS scan in both cases was started when the solvent had completely eluted from the GC column. More recent studies on glass capillary column selection and performance on these types of samples have been conducted by Lin et al. (*14, 22*).

The data system acquisition parameters used in this study were: computer software program, IFSS (*23*); preamplifier, 10^{-8} A/V; mass range, 14–500 amu; integration time, 2 ms; samples/amu, 1; repeat count before checking lower threshold, 2; maximum repeat count, 5; lower threshold, 3; upper threshold, 1; second disk?, yes; scan time, 2.5 s (average).

Data was collected for approximately 2.5 h and stored temporarily on disk memory units. Special computer software was required to process the data from System 150 GC/MS runs having more than 2047 spectra. It was also necessary to revise the System 150 software to copy these long data files from a completely filled disk to magnetic tape for storage. The data from each GC/MS run in the form of total ion chromatograms (TIC), mass chromatograms (MC), and spectra were displayed on Tektronix CRT data terminals or obtained as hard copy output on a digital plotter. Magnetic tape was used for archival storage of data.

Compound Identification. The following criteria were required of all confirmation studies: (1) The mass spectrum of the unknown compound matched the mass spectrum of a

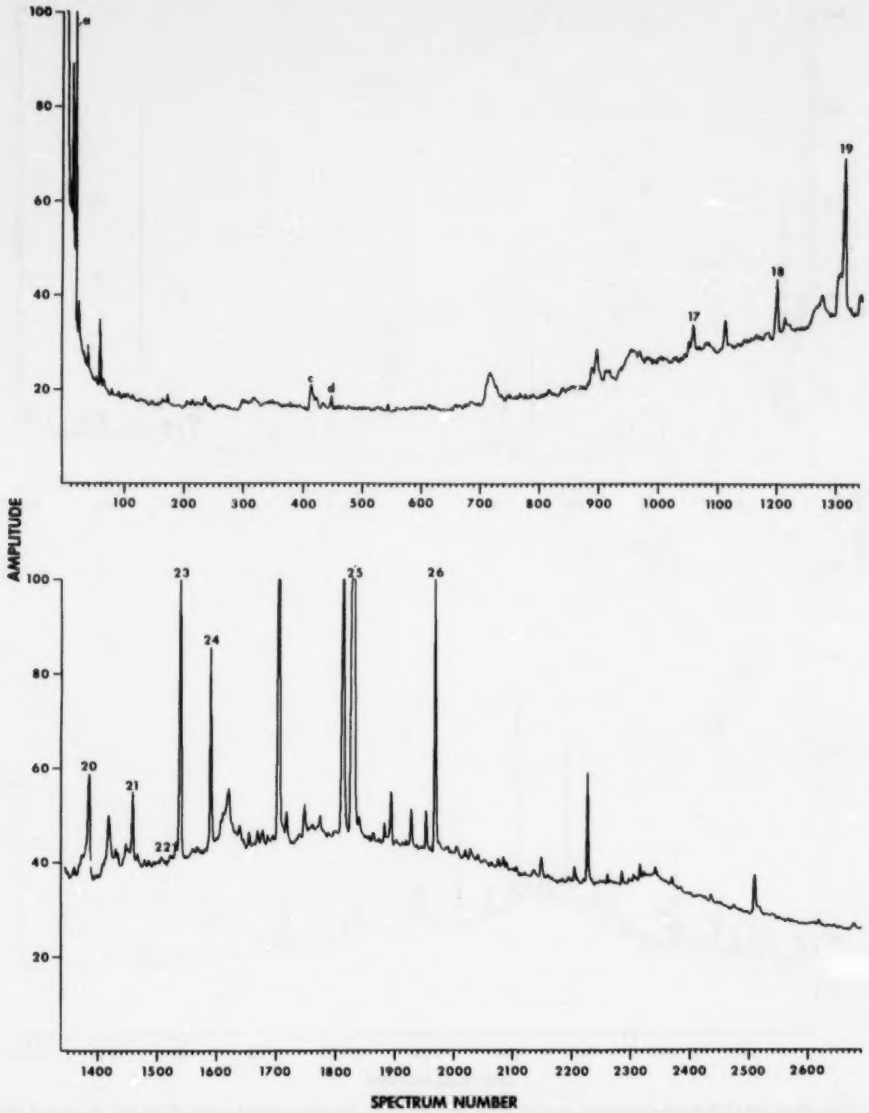

Figure 3. Total ion chromatogram of the same extract as in Figure 2 on SE30. Numbers identify the following GC peaks: (17) dimethyl phthalate; (18) diethyl phthalate; (19 and 20) *N*-ethyltolylsulfonamide isomers; (21) diisobutyl phthalate; (22) 9,10-anthraquinone; (23) dibutyl phthalate; (24) ethyl palmitate; (25) benzyl butyl phthalate; (26) dioctyl phthalate. The large peak at spectrum no. 1703 is bis(butoxyethoxyethoxy)methane, a major contaminant of the RO process (*1, 34*). The peak immediately preceding no. 25 is an unknown phthalate isomer. Peaks at spectra 2227 and 2509 are also unknown contaminants of the RO process (*1*)

standard compound run on the same instrument under the same GC/MS conditions. (2) The relative retention time (RRT) of the unknown compound agreed with that of a standard compound run under identical GC conditions. (3) The mass spectrum agreed with the spectrum found in the Cyphernetics MSSS or NIH/EPA/MSDC spectral matching systems (*24–26*). (4) The mass spectrum agreed with the mass spectrum found in the literature (*27, 28*). (5) The mass spectrum agreed with our interpretation. (In cases where this was the only confirmatory technique, an authentic standard was not available at the time, nor was there a spectrum in the computer data bases or the literature.) If a compound meets criteria 1–5 it is listed as a positive (P) identification (ID) in Table I. If it meets criteria 3–5, its identification is listed as tentative (T).

A compound was considered "detected" in the extracts when the same compound, as determined by GC/MS data, either was absent in the blanks or was significantly above the level of that found in the blank extracts. In addition to these procedural blanks, previous studies by Kopfler et al. (*1*), which reported specific organic compounds that leached from the components of the RO concentrator unit into water samples during the concentration, were also taken into account.

After the mass spectra of all obvious GC peaks were obtained from each RGC, it was apparent that many spectra were contaminated by interfering ions from adjacent or underlying GC peaks that had not been initially detected. A computerized technique (*29*) of extracting specific ions from a total ion current chromatogram was used extensively in this study to resolve the effect of overlapping GC peaks (*29–31*). This technique allowed one to isolate and enhance single compounds and classes of compounds having characteristic ions different from background interferences.

Results and Discussion

The results of the foregoing experiments are described in the following paragraphs. The notations CE, A, MA, BN, and others in Table I, under the subheading fraction found in, refer

Table I. Organic Compounds in the Diethyl Ether Extract of the RO Concentrate

compound	formula	mol wt[a]	fraction found in				relative size (1–10)	T or P ID
			CE	A	MA	BN0–7		
		Ketones and Aldehydes						
*chloroacetone[d]	C_3H_5OCl	92	CE	A		BN0	<1	P
butanedione, 2,3-	$C_4H_6O_2$	86		A			<1	P
*chloro-2-butanone, 3-	C_4H_7OCl	106	CE	A	MA		<1	P
*chlorobutanone isomer	C_4H_7OCl	106				BN0	<1	T
methyl ethyl ketone[b]	C_4H_8O	72	CE				<1	P
furaldehyde, 2-	$C_5H_4O_2$	96		A		BN6	<1	P
methylbutenal isomer	C_5H_8O	84	CE		MA		1	T
methyl-2-butenal, 2-	C_5H_8O	84	CE	A	MA	BN0	<1	P
methyl propenyl ketone, *cis*-	C_5H_8O	84	CE	A			4	P
methyl isopropenyl ketone	C_5H_8O	84	CE			BN0	1	P
methyl propenyl ketone, *trans*-	C_5H_8O	84	CE				1	P
methyl-3-buten-2-one, 3-	C_5H_8O	84			MA		<1	P
pent-2-en-4-one	C_5H_8O	84		A			1	P
cyclopentanone[b]	C_5H_8O	84		A			<1	P
pentanone, 2-	$C_5H_{10}O$	86	CE		MA		<1	P
methyl-2-butanone, 3-[b]	$C_5H_{10}O$	86	CE				<1	P
hydroxy-3-methyl-2-butanone, 3-	$C_5H_{10}O_2$	102		A			<1	P
hydroxy-3-methyl-2-butanone, 4-	$C_5H_{10}O_2$	102		A			<1	P
formylcyclopentene, 1-	C_6H_6O	96				BN5	<1	P
dimethylcyclobutenone	C_6H_8O	96				BN0	<1	P
*chlorocyclohexanone, 2-	C_6H_9OCl	132		A		BN0, 2, 4, 5	<1	P
methylpentenal isomer	$C_6H_{10}O$	98			MA		<1	T
cyclohexanone[b]	$C_6H_{10}O$	98	CE				<1	P
methyl-1-penten-3-one, 4-	$C_6H_{10}O$	98				BN5	<1	P
methylcyclopentanone (2 isomers)	$C_6H_{10}O$	98		A		BN6	<1	T
methyl-3-penten-2-one, 4-	$C_6H_{10}O$	98	CE	A		BN0	<1	P
*chloro-2-hexanone, 3-	$C_6H_{11}OCl$	134		A			<1	T
hexanone, 2-	$C_6H_{12}O$	100	CE		MA	BN0, 2, 6, 7	<1	P
hexanone, 3-	$C_6H_{12}O$	100	CE	A		BN0, 2, 6, 7	<1	P
hexanal	$C_6H_{12}O$	100	CE	A		BN0, 2, 4, 5	<1	P
diacetone alcohol	$C_6H_{12}O_2$	116		A			<1	P
benzaldehyde[b]	C_7H_6O	106	CE	A		BN0, 5, 6	<1	P
methylcyclohex-1-en-3-one, 1-	$C_7H_{10}O$	110		A			<1	P
*chloro-3-hepten-2-one, 4-	$C_7H_{11}OCl$	146				BN6	<1	T
methylcyclohexanone, 4-	$C_7H_{12}O$	112				BN4, 5	<1	P
dimethyl-3-penten-2-one, 3,4-	$C_7H_{12}O$	112		A	MA		<1	P
methylhex-3-en-2-one isomer	$C_7H_{12}O$	112		A			<1	P
heptanone, 2-	$C_7H_{14}O$	114	CE				<1	P
dimethyl-3-pentanone, 2,4-	$C_7H_{14}O$	114				BN6	<1	P
methyl-2-hexanone, 5-	$C_7H_{14}O$	114		A		BN0	<1	P
acetophenone[b]	C_8H_8O	120				BN6	<1	P
methylhepta-2,4-dione isomer	$C_8H_{14}O_2$	142				BN0	<1	T
methylheptanone isomer	$C_8H_{16}O$	128		A			<1	T
methyl-2-heptanone, 3-	$C_8H_{16}O$	128		A			<1	P
methyl-2-heptanone, 5-	$C_8H_{16}O$	128		A			<1	P
octanone, 3-	$C_8H_{16}O$	128		A			<1	P
methylacetophenone[b]	$C_9H_{10}O$	134	CE			BN0	<1	P
isophorone[b,c]	$C_9H_{14}O$	138	CE			BN0	<1	P
dimethyl-5-heptenal, 2,6-	$C_9H_{16}O$	140		A			<1	P
methyloctanone	$C_9H_{18}O$	142		A			<1	T
dimethylacetophenone	$C_{10}H_{12}O$	148		A		BN0, 7	<1	P
myrtenal	$C_{10}H_{14}O$	150		A			<1	P
tetramethyl-2-cyclohexenone (2 isomers)	$C_{10}H_{16}O$	152				BN0, 7	<1	T
trimethyl-1-cyclohexane-4-carboxaldehyde, 1,2,4-	$C_{10}H_{16}O$	152				BN6	<1	T
dodecane-6,7-dione	$C_{12}H_{22}O_2$	198		A			<1	P
fluorenone, 9-	$C_{13}H_8O$	180				BN5	<1	P
phenylbenzaldehyde, 4-	$C_{13}H_{10}O$	182				BN2	<1	P
tetramethylindanone isomer	$C_{13}H_{16}O$	188				BN2	<1	T
anthraquinone, 9,10-	$C_{14}H_8O_2$	208	CE				<1	P
di-*tert*-butyl-*p*-benzoquinone, 2,6-[b]	$C_{14}H_{20}O_2$	220				BN5	<1	P
tetradecanone (2 isomers)	$C_{14}H_{28}O$	212				BN5	<1	T

Table I. Continued

compound	formula	mol wt[a]	fraction found in CE	A	MA	BN0–7	relative size (1–10)	T or P ID
methyl 2-benzoylbenzoic acid	$C_{15}H_{12}O_3$	240		A			<1	P
methyl 4-benzoylbenzoic acid	$C_{15}H_{12}O_3$	240		A			<1	P
dibutyldimethylbicyclohexanone isomer	$C_{16}H_{28}O$	236	CE			BN0, 6, 7	<1	T
epidehydroabietal, 4-	$C_{20}H_{28}O$	284				BN2	<1	P
		Alcohols						
*dichloro-2-propanol, 1,1-	$C_3H_6OCl_2$	128				BN4	<1	P
acetol	$C_3H_6O_2$	74		A			<1	P
methyl acetyl carbinol	$C_4H_8O_2$	88		A			1	P
butanol	$C_4H_{10}O$	74	CE	A		BN5, 6, 7	<1	P
*chlorobutanol isomer	C_4H_9OCl	108	CE		MA	BN6	<1	T
*chloropenten-5-ol	C_5H_9OCl	120		A			<1	T
methyl-2-butenol, 2-	$C_5H_{10}O$	86	CE	A	MA	BN0, 6	1	P
methyl-2-butenol, 3-	$C_5H_{10}O$	86	CE	A	MA		<1	P
amyl alcohol, *tert*-	$C_5H_{12}O$	88			MA		<1	P
methyl-2-butanol, 2-[b]	$C_5H_{12}O$	88	CE				<1	P
pentanol	$C_5H_{12}O$	88	CE	A		BN0, 7	<1	P
methyl-2-butanol, 3-	$C_5H_{12}O$	88	CE				<1	P
phenol[b]	C_6H_6O	94		A		BN6	<1	P
dimethylbuten-3-ol, 2,3-	$C_8H_{12}O$	100		A			<1	P
*chloro-3-hexanol, 6-	$C_6H_{13}OCl$	136	CE	A			<1	T
dimethylbutanol isomer	$C_6H_{14}O$	102	CE				<1	T
hexanol, 1-	$C_6H_{14}O$	102	CE			BN7	<1	P
hexanol, 2-	$C_6H_{14}O$	102	CE	A		BN0, 2, 6, 7	<1	P
hexanol, 3-	$C_6H_{14}O$	102				BN0, 2, 6, 7	<1	P
butoxyethanol, 2-[b]	$C_6H_{14}O_2$	118	CE	A	MA	BN0, 7	<1	P
isopropoxy-2-propanol, 1-	$C_6H_{14}O_2$	118		A			<1	P
diethylene glycol monoethyl ether	$C_6H_{14}O_3$	134	CE		MA	BN7	<1	P
diethylene glycol dimethyl ether	$C_6H_{14}O_3$	134	CE	A			<1	P
benzyl alcohol	C_7H_8O	108	CE	A			<1	P
heptenol, 2-	$C_7H_{14}O$	114				BN5	<1	P
heptanol, 1-	$C_7H_{16}O$	116				BN7	<1	P
butoxy-2-propanol, 1-	$C_7H_{16}O_2$	132		A		BN7	<1	P
dipropylene glycol methyl ether	$C_7H_{16}O_3$	148	CE				<1	P
dimethylphenol, 2,4-[b,c]	$C_8H_{10}O$	122	CE	A			<1	P
dimethylcyclohexanol, 2,6-	$C_8H_{16}O$	128				BN0	<1	P
methyl-3-ethyl-3-pentanol, 2-	$C_8H_{18}O$	130			MA		<1	P
octanol	$C_8H_{18}O$	130	CE				<1	P
octanol, 3-	$C_8H_{18}O$	130	CE	A			<1	P
butoxyethoxy)ethanol, 1-(2-[b]	$C_8H_{18}O_3$	162	CE	A		BN7	1	P
butoxyethoxy)ethanol, 2-(2-	$C_8H_{18}O_3$	162	CE		MA	BN0, 6	9	P
ethoxypropoxypropanol (2 isomers)	$C_8H_{18}O_3$	162		A			<1	T
tripropylene glycol	$C_9H_{20}O_4$	192		A			<1	P
borneol[b]	$C_{10}H_{18}O$	154	CE			BN0, 6, 7	<1	P
terpineol, α-	$C_{10}H_{18}O$	154				BN0, 7	<1	P
decanol	$C_{10}H_{22}O$	158				BN0	<1	P
dimethoxy-3-hydroxy-2,4-dimethylhexane, 1,5-	$C_{10}H_{22}O_3$	190		A			<1	T
triethylene glycol butyl ether (3 isomers)	$C_{10}H_{22}O_4$	206	CE	A		BN7	<1	T
tripropylene glycol methyl ether (4 isomers)	$C_{10}H_{22}O_4$	206		A		BN7	<1	T
butylhydroxyanisole isomer	$C_{11}H_{16}O_2$	180				BN0, 6	<1	T
tetradecanol	$C_{14}H_{30}O$	214				BN6	<1	P
di-*tert*-butyl-4-methylphenol, 2,6-	$C_{15}H_{24}O$	220				BN6	<1	P
dibutylhydroxyanisole (2 isomers)	$C_{15}H_{24}O_2$	236	CE			BN0, 6, 7	1	T
farnesol	$C_{15}H_{26}O$	222				BN5	<1	T
di-*tert*-butylethylphenol isomer	$C_{16}H_{26}O$	234		A			<1	T
hexadecanol, 1-	$C_{16}H_{34}O$	242	CE				<1	P
octadecanediol, 1,2-	$C_{18}H_{38}O_2$	286				BN5	<1	P
nonadecanol	$C_{19}H_{40}O$	284				BN4	<1	P
		Ethers						
dioxolane, 1,3-	$C_3H_6O_2$	74	CE				<1	P
trioxane, 1,3,5-[b]	$C_3H_6O_3$	90		A			<1	P
*chloroethyl)ether, bis(2-[b,c]	$C_4H_8OCl_2$	142	CE				<1	P

Table I. Continued

compound	formula	mol wt[a]	fraction found in CE	A	MA	BN0–7	relative size (1–10)	T or P ID
dimethoxyethane	$C_4H_{10}O_2$	90	CE				<1	P
*dichloro-4-methoxy-1-butene, 1,1-	$C_5H_8OCl_2$	154	CE				<1	P
ethoxypropene, 2-	$C_5H_{10}O$	86				BN2	<1	P
dimethylfuran, 2,4-	C_6H_8O	96	CE			BN2	<1	P
dihydro-2-methylpyran, 5,6-	$C_6H_{10}O$	98			MA	BN0	<1	T
dimethyltetrahydrofuran, 3,4-	$C_6H_{12}O$	100				BN7	<1	P
ethyl *sec*-butyl ether	$C_6H_{14}O$	102	CE				<1	P
methoxyethyl)ether, bis(2-	$C_6H_{14}O_3$	134		A			<1	P
propyl butyl ether	$C_7H_{16}O$	116		A			<1	P
methyl-5-isopropylfuran, 2-	$C_8H_{12}O$	124	CE				<1	P
butyl ether, *sec*-	$C_8H_{18}O$	130				BN7	<1	P
butyl ether	$C_8H_{18}O$	130				BN7	<1	P
ethoxyethyl)ether, bis(2-	$C_8H_{18}O_3$	162				BN0	<1	P
pentylfuran, 2-	$C_9H_{14}O$	138	CE	A		BN7	<1	P
dibenzofuran	$C_{12}H_8O$	168				BN2	<1	P
diphenyl ether	$C_{12}H_{10}O$	170		A		BN0, 4	<1	P
*chlorophenyl phenyl ether, 2-[c]	$C_{12}H_{10}OCl$	204				BN2	<1	P
methyl-*m*-methoxynaphthalene	$C_{12}H_{12}O$	172			MA		<1	P
		Acids						
*trichloroacetic acid	$C_2HO_2Cl_3$	162				BN6	<1	P
acetic acid[b]	$C_2H_4O_2$	60	CE			BN6	<1	P
succinic acid	$C_4H_6O_4$	118			MA		<1	P
oxopentanoic acid, 4-	$C_5H_8O_3$	116			MA		<1	P
pentanoic acid	$C_5H_{10}O_2$	102			MA		<1	P
methylpentanoic acid, 3-	$C_6H_{12}O_2$	116			MA		<1	P
hexanoic acid	$C_6H_{12}O_2$	116	CE		MA		<1	P
benzoic acid[b]	$C_7H_6O_2$	122		A	MA		<1	P
salicylic acid[b]	$C_7H_6O_3$	138			MA		<1	P
methyl-4-formylpentanoic acid, 4-	$C_7H_{12}O_3$	144	CE				<1	T
heptanoic acid	$C_7H_{14}O_2$	130	CE	A	MA		<1	P
phenylacetic acid	$C_8H_8O_2$	136			MA		<1	P
toluic acid, *m*-	$C_8H_8O_2$	136			MA		<1	P
toluic acid, *p*-	$C_8H_8O_2$	136			MA		<1	P
methoxybenzoic acid, *p*-	$C_8H_8O_3$	152			MA		<1	P
methylhept-6-enoic acid, 6-	$C_8H_{14}O_2$	142			MA		<1	T
octanedioic acid, 1,8-	$C_8H_{14}O_4$	174	CE				<1	P
octanoic acid	$C_8H_{16}O_2$	144	CE		MA		<1	P
ethylhexanoic acid, 2-	$C_8H_{16}O_2$	144			MA		<1	P
dimethylbenzoic acid, 3,5-	$C_9H_{10}O_2$	150			MA		<1	P
phenylpropanoic acid, 2-	$C_9H_{10}O_2$	150			MA		<1	P
phenylpropanoic acid, 3-	$C_9H_{10}O_2$	150			MA		<1	P
oxononanoic acid, 4-	$C_9H_{16}O_3$	172			MA		<1	P
azelaic acid	$C_9H_{16}O_4$	188			MA		<1	P
methyloctanedioic acid, 3-	$C_9H_{16}O_4$	188			MA		<1	P
trimethylhexanoic acid, 3,5,5-	$C_9H_{18}O_2$	158			MA		<1	P
nonanoic acid	$C_9H_{18}O_2$	158	CE	A	MA		<1	P
decanedioic acid	$C_{10}H_{18}O_4$	202			MA		<1	P
decanoic acid	$C_{10}H_{20}O_2$	172		A	MA		<1	P
undecanedioic acid, 1,11-	$C_{11}H_{20}O_4$	216			MA		<1	P
undecanoic acid	$C_{11}H_{22}O_2$	186			MA		<1	P
xylylbutanoic acid (2 isomers)	$C_{12}H_{16}O_2$	192	CE		MA	BN5, 6	<1	T
dodecanedioic acid	$C_{12}H_{22}O_4$	230			MA		<1	P
dodecanoic acid	$C_{12}H_{24}O_2$	200	CE	A	MA		<1	P
tridecanoic acid	$C_{13}H_{26}O_2$	214			MA		<1	P
tetradecanoic acid	$C_{14}H_{28}O_2$	228		A	MA		<1	P
methylheptan-4′-on-2′-yl)-cyclohex-1-ene carboxylic acid, 4-(6′-	$C_{15}H_{24}O_3$	252			MA		<1	T
pentadecanoic acid	$C_{15}H_{30}O_2$	242			MA		<1	P
hexadecenoic acid, *cis*-	$C_{16}H_{30}O_2$	254	CE				<1	T
hexadecanoic acid	$C_{16}H_{32}O_2$	256	CE	A	MA		<1	P
heptadecanoic acid	$C_{17}H_{34}O_2$	270			MA		<1	P
oleic acid	$C_{18}H_{34}O_2$	282			MA		<1	P

Table I. Continued

compound	formula	mol wt[a]	fraction found in CE	A	MA	BN0–7	relative size (1–10)	T or P ID
octadecanoic acid	$C_{18}H_{36}O_2$	284	CE	A	MA		<1	P
dehydroabietic acid	$C_{20}H_{28}O_2$	300			MA		<1	P
	Esters (Including Lactones)							
methyl formate	C_2H_4O	60				BN7	<1	P
butyrolactone, γ-	$C_4H_6O_2$	86	CE	A		BN0	<1	T
ethylene diformate	$C_4H_6O_4$	118		A			<1	P
*ethyl chloroacetate	$C_4H_7O_2Cl$	122	CE	A			<1	P
valerolactone, δ-	$C_5H_8O_2$	100		A			<1	T
methyl methacrylate[b]	$C_5H_8C_2$	100				BN5	<1	P
dimethyl malonate	$C_5H_8O_4$	132				BN6	<1	P
ethyl propanoate	$C_5H_{12}O_2$	102	CE				<1	P
caprolactone, δ-	$C_6H_{10}O_2$	114	CE	A			1	T
caprolactone, γ-	$C_6H_{10}O_2$	114	CE	A		BN0	1	T
vinyl butyrate	$C_6H_{10}O_2$	114				BN0	<1	P
ethoxybutanolide, 4-	$C_6H_{10}O_3$	130	CE				<1	T
heptanolactone, γ-	$C_7H_{12}O_2$	128	CE				<1	T
heptanolactone, ε-	$C_7H_{12}O_2$	128				BN0	<1	T
isopentenyl acetate isomer	$C_7H_{12}O_2$	128	CE	A			<1	T
diethyl malonate	$C_7H_{12}O_4$	160	CE				<1	P
methyl butyl acetate, 2-	$C_7H_{14}O_2$	130		A			<1	P
isopropyl ethoxyacetate	$C_7H_{14}O_3$	146		A			<1	P
ethyl isopropoxyacetate	$C_7H_{14}O_3$	146	CE				<1	P
ethyl orthoformate	$C_7H_{16}O_3$	148			MA		<1	P
phthalide	$C_8H_6O_2$	134	CE			BN6	<1	P
methyl benzoate[b]	$C_8H_8O_2$	136			MA		<1	P
octalactone, γ-	$C_8H_{14}O_2$	142				BN0	<1	T
octalactone, δ-	$C_8H_{14}O_2$	142				BN0	<1	T
ethyl 2-ethyl-3-oxobutanoate	$C_8H_{14}O_3$	158			MA		<1	T
butyl butanoate (2 isomers)	$C_8H_{16}O_2$	144	CE			BN0, 6, 7	<1	T
methyl heptanoate	$C_8H_{16}O_2$	144	CE				<1	P
methylphthalide (2 isomers)	$C_9H_8O_2$	148				BN0, 6	<1	T
methyl 3-methylbenzoate[b]	$C_9H_{10}O_2$	150	CE				<1	P
methyl toluate (2 isomers)	$C_9H_{10}O_2$	150			MA		<1	P
glycerol triacetate	$C_9H_{14}O_6$	218				BN0	<1	P
nonalactone, γ-	$C_9H_{16}O_2$	156				BN7	<1	T
ethyl phenylacetate	$C_{10}H_{12}O_2$	164	CE		MA		<1	P
propyl benzoate isomer	$C_{10}H_{12}O_2$	164			MA		<1	T
decalactone, γ-	$C_{10}H_{18}O_2$	170				BN0	<1	T
decalactone, δ-	$C_{10}H_{18}O_2$	170	CE	A			<1	T
ethyl octanoate	$C_{10}H_{20}O_2$	172	CE				<1	P
ethylhexyl acetate, 2-	$C_{10}H_{20}O_2$	172		A			<1	P
octyl acetate	$C_{10}H_{20}O_2$	172		A			<1	T
butyl benzoate	$C_{11}H_{14}O_2$	178				BN4	<1	P
butyl salicylate	$C_{11}H_{14}O_3$	194		A			<1	P
dihydroactinidiolide	$C_{11}H_{16}O_2$	180	CE	A		BN0, 7	<1	T
amyl benzoate	$C_{12}H_{16}O_2$	192				BN4	<1	P
*benzyl *p*-chlorobenzoate	$C_{14}H_{11}O_2Cl$	246				BN0	<1	P
benzyl benzoate	$C_{14}H_{12}O_2$	212				BN0, 5	<1	P
methyl tridecanoate	$C_{14}H_{28}O_2$	228			MA	BN4	<1	P
methyl tetradecanoate	$C_{15}H_{30}O_2$	242			MA	BN7	<1	P
benzyl 3-phenylpropionate	$C_{16}H_{16}O_2$	240			MA	BN0	<1	T
trimethylpenta-1,3-diol diisobutyrate, 2,2,4-	$C_{16}H_{30}O_4$	286				BN0, 6, 7	<1	P
methylpentadecanoate	$C_{16}H_{32}O_2$	256			MA	BN7	<1	P
ethyl myristate	$C_{16}H_{32}O_2$	256				BN5	<1	P
phenyl naphthoate	$C_{17}H_{12}O_2$	248				BN5	<1	P
dibutyl azelate isomer	$C_{17}H_{32}O_4$	300	CE			BN0	<1	T
methyl hexadecanoate[b]	$C_{17}H_{34}O_2$	270	CE		MA	BN0, 4,5, 7	<1	P
diethylene glycol dibenzoate	$C_{18}H_{18}O_5$	314		A		BN0, 2, 4	<1	P
butyl phthalyl butyl glycolate	$C_{18}H_{26}O_5$	322	CE			BN0, 6	<1	P
dibutyl sebacate	$C_{18}H_{34}O_4$	314				BN0	<1	P
methyl heptadecanoate	$C_{18}H_{36}O_2$	284			MA	BN0, 5, 7	<1	P

Table I. Continued

compound	formula	mol wt[a]	fraction found in				relative size (1–10)	T or P ID
			CE	A	MA	BN0–7		
ethyl palmitate[b]	$C_{18}H_{36}O_2$	284	CE	A		BN0, 4, 5	5	P
ethyl heptadecanoate	$C_{19}H_{38}O_2$	298				BN5	<1	P
methyl stearate[b]	$C_{19}H_{38}O_2$	298			MA		<1	P
methyl 4-hydroxyoctadecanoate	$C_{19}H_{38}O_3$	314				BN0	<1	T
ethyl stearate	$C_{20}H_{40}O_2$	312				BN5	<1	P
methyl dehydroabietate	$C_{21}H_{30}O_2$	314			MA	BN5	<1	P
dioctyl adipate[b]	$C_{22}H_{42}O_4$	370	CE			BN0, 6	<1	T
docosyl acetate	$C_{24}H_{48}O_2$	368				BN5	<1	T
dioctyl sebacate	$C_{26}H_{50}O_4$	426	CE			BN0	<1	P
	Hydrocarbons (Nonaromatic)							
*dichloroethylene isomer[b,c]	$C_2H_2Cl_2$	96			MA		<1	T
*bromo-1-chloroethane, 2-	C_2H_4BrCl	142				BN6, 7	<1	P
*dichloropropene, *cis*-1,3-[c]	$C_3H_4Cl_2$	110				BN6	<1	P
*trichloropropane, 1,1,1,-[b]	$C_3H_5Cl_3$	146	CE				<1	P
*trichloropropane, 1,2,3-[b]	$C_3H_5Cl_3$	146				BN0, 2	<1	P
*iodopropane	C_3H_7I	170	CE				<1	P
*chlorobutane isomer	C_4H_9Cl	92	CE				<1	T
*chloro-2-methyl-2-butene, 1-	C_5H_9Cl	104		A			<1	P
*chloro-3-methyl-2-butene, 2-	C_5H_9Cl	104		A			<1	P
*dichloro-2-methylbutane, 2,3-	$C_5H_{10}Cl_2$	140	CE	A		BN0	4	P
*bromopentane, 2-	$C_5H_{11}Br$	150				BN2	<1	P
*dichloroperfluoro-1,3,5-hexatriene, 1,6-	$C_6Cl_2F_6$	256				BN2	<1	T
bicyclo[3.1.0]hexane	C_6H_{10}	82				BN5	<1	P
*bromohexane isomer	$C_6H_{13}Br$	164		A			<1	T
methylcyclohexane	C_7H_{14}	98				BN0,2, 4, 5	<1	P
*methyldichlorocyclohexane (2 isomers)	$C_7H_{12}Cl_2$	166				BN0	<1	T
trimethyl-1-pentene, 2,2,4-	C_8H_{16}	112	CE				<1	P
methyl-3-ethyl-1-pentene, 2-	C_8H_{16}	112				BN2	<1	P
*dichloro-2-methyl-2-(1′-methylbutyl)cyclopropane, 1,1-	$C_9H_{16}Cl_2$	194		A	MA		<1	T
methyl-4-isopropyl-1,3-cyclohexadiene	$C_{10}H_{16}$	136				BN6	<1	T
undecane	$C_{11}H_{24}$	156				BN2	<1	P
dodecene isomer	$C_{12}H_{24}$	168				BN0	<1	T
dodecane[b]	$C_{12}H_{26}$	170				BN2	<1	P
tridecane[b]	$C_{13}H_{28}$	184				BN2	<1	P
tetradecane[b]	$C_{14}H_{30}$	198				BN2	<1	P
pentadecene	$C_{15}H_{30}$	210				BN6, 7	<1	P
pentadecane[b]	$C_{15}H_{32}$	212				BN2	<1	P
hexadecane[b]	$C_{16}H_{34}$	226	CE			BN2	<1	P
heptadecane[b]	$C_{17}H_{36}$	240		A		BN2	<1	P
octadecane[b]	$C_{18}H_{38}$	254		A		BN2	<1	P
nonadecane	$C_{19}H_{40}$	268	CE	A		BN2	<1	P
dimethyl-7-octylbicyclo[4.4.0]decane, 2,5-	$C_{20}H_{38}$	278		A			<1	T
eicosane[b]	$C_{20}H_{42}$	282				BN2	<1	P
docosane[b]	$C_{22}H_{46}$	310	CE				<1	P
didecylcyclohexane, 1,3-	$C_{26}H_{52}$	364				BN5	<1	T
	Aromatic Derivatives (Including PNAs and PCBs)							
*trichlorobenzene isomer[b,c]	$C_6H_3Cl_3$	180		A		BN2	<1	P
*benzyl chloride	C_7H_7Cl	126	CE				<1	P
*chlorotoluene isomer	C_7H_7Cl	126				BN0	<1	T
toluene[b,c]	C_7H_8	92	CE				<1	P
*chloro-2-propylbenzene, 1-	$C_9H_{11}Cl$	154				BN2	<1	P
trimethylbenzene isomer[b]	C_9H_{12}	120				BN0, 7	<1	P
naphthalene[b,c]	$C_{10}H_8$	128				BN0, 2	<1	P
methyl isopropylbenzene	$C_{10}H_{14}$	134				BN7	<1	P
butylbenzene isomer[b]	$C_{10}H_{14}$	134				BN7	<1	T
methylnaphthalene (2 isomers)[b]	$C_{11}H_{10}$	142				BN0, 2	<1	P
*hexachlorobiphenyl isomer[c]	$C_{12}H_4Cl_6$	358				BN2	<1	T
*pentachlorobiphenyl (7 isomers)[b,c]	$C_{12}H_5Cl_5$	324				BN2	<1	T
*tetrachlorobiphenyl (3 isomers)[b,c]	$C_{12}H_6Cl_4$	290				BN0, 2	<1	T
*trichlorobiphenyl (5 isomers)[b,c]	$C_{12}H_7Cl_3$	256				BN0, 2	<1	T

Table I. Continued

compound	formula	mol wt[a]	fraction found in CE	A	MA	BN0–7	relative size (1–10)	T or P ID
*dichlorobiphenyl isomer[c]	$C_{12}H_8Cl_2$	222				BN2	<1	T
dimethylnaphthalene, 1,3-[b]	$C_{12}H_{12}$	156				BN0, 2	<1	P
dimethylnaphthalene, 2,4-[b]	$C_{12}H_{12}$	156				BN0, 2	<1	P
dimethylnaphthalene (4 isomers)	$C_{12}H_{12}$	156				BN0, 2	<1	T
phenylcyclohexadiene isomer, 1-	$C_{12}H_{12}$	156				BN0	<1	T
ethylnaphthalene (2 isomers)	$C_{12}H_{12}$	156				BN2	<1	P
cyclohexylbenzene	$C_{12}H_{16}$	160				BN2, 5, 6	<1	P
methylphenylpentane isomer	$C_{12}H_{18}$	162				BN6	<1	T
fluorene[c]	$C_{13}H_{10}$	166				BN2, 4	<1	P
trimethylnaphthalene (5 isomers)	$C_{13}H_{14}$	170				BN0, 2	<1	T
isopropylnaphthalene (2 isomers)	$C_{13}H_{14}$	170				BN0, 2	<1	P
trimethyl-1,2,3,4-tetrahydronaphthalene, 1,1,6-	$C_{13}H_{18}$	174				BN2	<1	P
trimethyl-1,2,3,4-tetrahydronaphthalene, 1,4,6-	$C_{13}H_{18}$	174				BN2	<1	P
trimethyl-1,2,3,4-tetrahydronaphthalene, 2,5,8-	$C_{13}H_{18}$	174				BN2	<1	P
anthracene[b,c]	$C_{14}H_{10}$	178				BN0, 2, 4	<1	P
phenanthrene[c]	$C_{14}H_{10}$	178				BN0, 2,4	<1	P
*dichloro(chlorophenyl)ethane isomer	$C_{14}H_{10}Cl_4$	318		A			<1	T
methylfluorene (2 isomers)	$C_{14}H_{12}$	180				BN0, 4	<1	T
isopropylmethylnaphthalene	$C_{14}H_{16}$	184				BN2	<1	T
propylmethylnaphthalene	$C_{14}H_{16}$	184				BN2	<1	T
octylbenzene	$C_{14}H_{22}$	190				BN6	<1	P
methylanthracene isomer	$C_{15}H_{12}$	192				BN4	<1	T
methylphenanthrene isomer[b]	$C_{15}H_{12}$	192				BN4	<1	T
dimethylfluorene (6 isomers)	$C_{15}H_{14}$	194				BN4	<1	T
diphenylpropane (2 isomers)	$C_{15}H_{16}$	196				BN4	<1	T
dimethyl-4-isopropylnaphthene, 1,6-	$C_{15}H_{18}$	198				BN2	<1	T
pentyldecahydronaphthalene isomer	$C_{15}H_{28}$	208	CE				<1	T
pyrene[b,c]	$C_{16}H_{10}$	202				BN0, 4	<1	P
fluoranthene[c]	$C_{16}H_{10}$	202				BN0, 4	<1	P
dimethylphenanthrene, 2,5-	$C_{16}H_{14}$	206				BN4	<1	P
hexyldecahydronaphthalene isomer	$C_{16}H_{30}$	222	CE				<1	T
benzofluorene isomer	$C_{17}H_{12}$	216				BN4	<1	T
benzo-7-phenylbicyclo[2.2.1]hept-2-ene, 5,6-	$C_{17}H_{14}$	218				BN4	<1	T
benzophenylbicycloheptene (3 isomers)	$C_{17}H_{14}$	218				BN4	<1	T
benzfluoranthene, 2,13-[c]	$C_{18}H_{10}$	226				BN4	<1	P
dihydrobenz[*a*]anthracene	$C_{18}H_{14}$	230	CE				<1	P
diphenylbenzene, 1,2-	$C_{18}H_{14}$	230				BN4	<1	P
hexahydrobenz[*a*]anthracene isomer	$C_{18}H_{18}$	234				BN4	<1	T
phenylcyclohexylcyclohexane (2 isomers)	$C_{18}H_{26}$	242				BN0, 2	<1	T
dicyclohexylbenzene isomer	$C_{18}H_{26}$	242				BN2	<1	T
butyl-7-hexyl-1,2,3,4-tetrahydronaphthalene, 6-	$C_{20}H_{32}$	272				BN2	<1	T
butylhexyldecahydronaphthalene (3 isomers)	$C_{20}H_{38}$	278	CE			BN2	<1	T
butyl-8-hexyldecahydronaphthalene, 2-	$C_{20}H_{38}$	278	CE				<1	T
Phthalates and Anhydrides								
phthalic anhydride[b]	$C_8H_4O_3$	148	CE	A		BN6	<1	P
dimethyl phthalate[b,c]	$C_{10}H_{10}O_4$	194	CE		MA	BN0, 6, 7	1	P
methyl ethyl phthalate	$C_{11}H_{12}O_4$	208			MA		<1	P
methyl ethyl terephthalate	$C_{11}H_{12}O_4$	208			MA		<1	P
diethyl phthalate[b,c]	$C_{12}H_{14}O_4$	222	CE			BN0, 6, 7	2	P
methyl butyl phthalate	$C_{13}H_{16}O_4$	236			MA	BN6, 7	<1	P
methyl isobutyl phthalate	$C_{13}H_{16}O_4$	236			MA		<1	P
dibutyl phthalate[b,c]	$C_{16}H_{22}O_4$	278	CE	A		BN0, 2, 4, 6	6	P
diisobutyl phthalate[b]	$C_{16}H_{22}O_4$	278	CE			BN0, 6, 7	2	P
butyl isobutyl phthalate	$C_{16}H_{22}O_4$	278				BN0, 6, 7	<1	P
butyl benzyl phthalate[c]	$C_{19}H_{20}O_4$	312	CE	A		BN0, 2,4, 5, 6	10	P
dicyclohexyl phthalate	$C_{20}H_{26}O_4$	330	CE			BN0, 2, 4, 5, 6	1	P
dibenzyl phthalate	$C_{22}H_{18}O_4$	346	CE				<1	P
dioctyl phthalate[c]	$C_{24}H_{38}O_4$	390	CE	A		BN0, 2, 4, 5, 6	6	P

Table I. Continued

compound	formula	mol wt[a]	fraction found in				relative size (1–10)	T or P ID
			CE	A	MA	BN0–7		
bis(2-ethylhexyl) phthalate[b,c]	$C_{24}H_{38}O_4$	390				BN5	1	P
diisooctyl phthalate	$C_{24}H_{38}O_4$	390				BN5	<1	T
didecyl phthalate (5 isomers)	$C_{28}H_{46}O_4$	446				BN5	<1	T
	Compounds Containing Heteroatoms (S, P, N)							
nitromethane	CH_3O_2N	61	CE				<1	P
acetamide	C_2H_5ON	59	CE				<1	P
dimethyl sulfone	$C_2H_6O_2S$	94		A			<1	P
*chloropropanamide, 3-	C_3H_6ONCl	107		A		BN0	<1	T
dimethylformamide, *N,N*-	C_3H_7ON	73	CE			BN0	<1	P
sulfolane	$C_4H_8O_2S$	120	CE	A			<1	P
dimethylacetamide, *N,N*-	C_4H_9ON	87	CE				<1	P
diethyl sulfone	$C_4H_{10}O_2S$	122		A			<1	P
pyridine	C_5H_5N	79				BN0, 4, 6	<1	P
pentenenitrile, 4-	C_5H_7N	81		A			<1	P
ethoxypropionitrile, β-	C_5H_9ON	99		A	MA	BN0	<1	T
diethylformamide, *N,N*-	$C_5H_{11}ON$	101		A			<1	P
*trichloroaniline, 2,4,6-	$C_6H_4NCl_3$	195		A			<1	P
cyanopyridine, 2-	$C_6H_4N_2$	104		A			<1	T
benzothiadiazole	$C_6H_4N_2S$	136				BN5	<1	P
*dichloroaniline isomer	$C_6H_5NCl_2$	161		A			<1	T
methylpyridine, 2-	C_6H_7N	93	CE			BN0	<1	P
methylpyridine, 3-	C_6H_7N	93	CE			BN0	<1	P
methylpyridine isomer	C_6H_7N	93	CE				<1	T
trimethyl-2,4,6-trioxohexahydrotriazine, 1,3,5-[b]	$C_6H_9O_3N_3$	171	CE			BN0, 7	<1	P
caprolactam, ϵ-[b]	$C_6H_{11}ON$	113		A			1	P
acetylmorpholine, *N*-	$C_6H_{11}O_2N$	129	CE	A			1	P
*chloroethyl)phosphate, tris-(2-[b]	$C_6H_{12}O_4PCl_3$	284				BN6	1	P
pentan-3-one *o*-methyloxime isomer	$C_6H_{13}ON$	115		A			1	T
benzothiazole	C_7H_5NS	135	CE	A		BN0, 6, 7	1	P
hydroxybenzothiazole, 2-	C_7H_5ONS	151		A			1	P
dinitrotoluene, 2,6-[b,c]	$C_7H_6O_4N_2$	182				BN0, 5	1	P
dimethylpyridine (3 isomers)	C_7H_9N	107				BN0	1	T
toluene-4-sulfonamide	$C_7H_9O_2NS$	171		A			1	P
isopropylthiophene, 2-	$C_7H_{10}S$	126				BN0	1	T
methylethylsuccinimide	$C_7H_{11}O_2N$	141		A			1	T
benzyl cyanide	C_8H_7N	117				BN0, 5, 6	1	P
methylthiobenzothiazole, 2-	$C_8H_7NS_2$	181				BN5	1	P
acetanilide	C_8H_9ON	135				BN7	1	P
trimethylpyridine, 2,4,6-	$C_8H_{11}N$	121				BN0	1	P
methyl-5-ethylpyridine, 2-[b]	$C_8H_{11}N$	121				BN0	1	P
dimethylaniline isomer	$C_8H_{11}N$	121				BN7	1	T
dimethylbenzenesulfonamide, 3,4-	$C_8H_{11}O_2NS$	185		A			1	T
methylphthalimide, *N*-	$C_9H_7O_2N$	161			MA		1	P
dibutylformamide, *N,N*-	$C_9H_{19}ON$	157	CE			BN0, 7	1	P
methylquinoline	$C_{10}H_9N$	143				BN0	1	T
butylbenzenesulfonamide	$C_{10}H_{15}O_2NS$	213				BN0	1	P
phenylpyridine	$C_{11}H_9N$	155			MA		1	P
carbazole	$C_{12}H_9N$	167				BN4	1	T
dibutylthiophene isomer	$C_{12}H_{20}S$	196				BN0, 6, 7	1	T
dodecanamide	$C_{12}H_{25}ON$	199				BN0, 7	1	P
tributyl phosphate[b]	$C_{12}H_{27}O_4P$	266	CE			BN0, 6, 7	1	P
(phenylene)bis[2-phenyldiazene], 1,1′-isomer	$C_{18}H_{14}N_4$	286				BN0	1	T
triphenyl phosphate[b]	$C_{18}H_{15}O_4P$	326				BN0	1	P

[a] The molecular weights are calculated on the basis of the integral masses of the most abundant isotopes. [b] In case of a single compound, it was reported previously in drinking water. In case of numbers of isomers, only one isomer was previously reported in drinking water. [c] Compound appeared in the EPA Consent Decree list of toxic pollutants (*33*). [d] Halogenated compounds are marked with an asterisk.

to the extracts, fractions, or partitions designated in Figure 1. Using this fractionation and partitioning scheme, followed by glass capillary gas chromatography/mass spectrometry, we were able to identify 460 of the more than 700 compounds detected in the extracts and fractions. The majority of these had not been previously reported in drinking water (*32*). The

compounds appearing in Table I are arranged according to class or classes and listed by increasing carbon number. The halogenated compounds are preceded by an asterisk (*). The following information is also given in Table I: molecular formula and weight, the fraction or fractions in which the compound was identified, positive or tentative identification, and the relative abundance of the compound as it appeared in the unpartitioned diethyl ether extract. The relative abundance is based on a scale of 1 to 10 with 10 being the highest concentration. It was impossible to determine the relative abundances in other fractions since some were combined in order to get enough material to give detectable amounts for identifiable mass spectra. So even though most of the compounds were identified after further partitioning of the diethyl ether extract, the relative abundance of compounds in this unpartitioned fraction is representative. Since this research was undertaken initially to validate methods for the separation and identification of compounds in complex mixtures and to provide drinking water concentrates large enough (about 1 g of chemical contaminants) for toxicological studies, the main effort was qualitative analysis. However, it is estimated (based on the level of internal standards and standards used for confirmation) that most of the contaminants, with the exception of some plasticizers, were present in the original water at levels less than 10 ppb.

The organics identified in the unpartitioned fraction represented only a small portion of the total organics identified in the partitioned fractions. Many of the chromatographic peaks were poorly resolved due to naturally occurring background interferences which were acidic, making resolution of adjacent GC peaks and separation of overlapping spectra fairly difficult and at many times impossible. Long, broad tailing GC peaks tended to overshadow and obscure those contaminants which were present in small concentrations or those which did not chromatograph so well on the particular column used during the GC/MS run. A subsequent extraction with 5% NaOH removed much of the acidic background from the diethyl ether fraction, leaving cleaner, better resolved GC peaks which could more easily be identified in the base/neutral fraction.

The few chromatographic peaks which did stand out well-defined above the background of polar humps were identified as butoxyethoxyethane, tributyl phosphate, benzothiazole, ethyl palmitate, and a number of phthalates. A potentially harmful herbicide (1,3,5-trimethyl-2,4,6-trioxohexahydrotriazine) and several potentially carcinogenic PNAs (7,12-dihydrobenz[a]anthracene and 9,10-anthraquinone) were identified among the rounded humps on the background, while the PCBs and most of the PNA peaks remained indistinguishable. Unless specific computer searching for a particular characteristic molecular ion had been done, these PNAs and PCBs and many of the chlorinated species would never have been found in the unpartitioned ether-soluble fraction. Separation into the acid and base/neutral fractions and subsequent partitioning of the base/neutral fraction on a silica gel microcolumn selectively separated PNAs and PCBs and other organic groups into individual partitions, permitting sharper peaks, cleaner base lines, and fewer compounds present in a single GC/MS run. Figures 2 and 3 show the complexity of this fraction chromatographed on two different columns. Despite the use of high-resolution capillary columns, less than 100 GC peaks were resolved enough to make positive identification. The SP1000 column had limited use for high molecular weight polar compounds, but was quite adequate in resolving lower molecular weight polar compounds. The retention time (t_R) of dibutyl phthalate on SP1000 exceeded 2 h. The t_R's of dibutyl phthalate and dioctyl phthalate were both less than 2 h on SE30. In general, a large number of the halogenated species and most of the aromatics seen in the partitioned fractions were hidden beneath the rounded peaks and the acidic background slug of material. Relatively few free organic acids were found in the acid fraction; C_6–C_{10}, C_{12}, C_{14}, and C_{18} straight-chained carboxylic acids and benzoic acid were identified as broad humps on the SE30 column. (They appeared even broader on the SP1000 column.) Because carboxylic acids are often difficult to chromatograph, a portion of the acid fraction was methylated with diazomethane in an attempt to distinguish more of the acidic contaminants as their methyl ester derivatives. In the GC/MS runs involving the methylated fraction, many more aromatic, straight-chained, branched acids and diacids were identified as methyl esters. Higher molecular weight saturated and unsaturated carboxylic acids with even carbon numbers (C_{14}, C_{16}, C_{18}) tended to be the most predominant acids in the sample.

Following partitioning of the base/neutral extract on the silica gel microcolumn, subfractions 1, 2, and 3, which were eluted with 0.5, 1.0, and 4 mL of hexane, respectively, were combined for GC/MS analysis. The analysis revealed the presence of a larger number of PCBs than had been resolved in the GC/MS analysis of the unpartitioned base and neutral fraction. Isomers identified in this subfraction included two dichloro-, four trichloro-, two tetrachloro-, six pentachloro-, and one hexachlorobiphenyl, which gives some indication of its potentially carcinogenic nature. A large percentage of the compounds identified in this fraction were aromatic or cyclic hydrocarbons.

The fourth subfraction (4 mL of hexane/benzene, 1:1) was characterized primarily by a large number of polynuclear aromatics (PNAs) and benzene derivatives which are potentially carcinogenic to humans. This PNA fraction, together with the PCB-containing partition (BN1-3), contained the highest number of contaminants with potential carcinogenic character. Many of the PNAs detected in this sample are labeled as nonspecific isomers in Table I. Unfortunately, even after running authentic standards on the GC/MS using the same capillary columns, we were not able to confirm some PNA isomers of equal molecular weight having very close relative t_R's, because the original GC/MS data was acquired using a variable scan time data acquisition program (IFSS), and our data system did not provide retention time information. Using such a program to determine relative t_R requires that sample and standard solutions contain approximately the same level of contaminants. The level of PNAs in this fraction was less than 5 ng per component.

Summary and Conclusions

It is apparent that an attempt to identify trace amounts of organic compounds of various polarities in a drinking water sample such as the diethyl ether extract of the RO concentrate would result in very few confirmations by GC/MS. We have demonstrated that partitioning a concentrate into acid, methylated acid, and base/neutral fractions is necessary in order to better resolve GC peaks for identification and quantification of individual components. Further cleanup of the base/neutral fraction was achieved by partitioning it on a silica gel microcolumn which was eluted with a range of solvents of diverse polarities: hexane, hexane/benzene (1:1), benzene, methylene chloride, and methanol.

Glass capillary GC/MS analysis of the resulting subfractions yielded an interesting array of compounds. A hexane subfraction revealed a predominance of PCBs, aromatics, chlorinated aromatics, and low molecular weight PNAs through anthracene. The hexane/benzene (1:1) subfraction was characterized by a large number of higher molecular weight PNAs and benzene derivatives.

In general, both GC columns (SE30 and SP1000) performed equally well on these complex mixtures. The SP1000 column was limited to organics that elute before dibutyl phthalate (2.5

h), whereas the SE30 column was able to elute organics with retention times beyond dioctyl phthalate (2 h). The acid fraction was the most difficult fraction to chromatograph; the free acids did not elute or separate readily on SP1000, but on SE30 broad GC humps were observed. Derivatization of the acids into their corresponding methyl esters resulted in numerous identifications which were not apparent in the acid fraction alone. Fractionation, derivatization, partitioning, and glass capillary gas chromatography/mass spectrometry of the diethyl ether extract effected the identification of 460 compounds of the more than 700 detected.

When examined for mutagenicity, the diethyl ether concentrate as a whole was tested. No one can predict which components caused the positive result in such a complex mixture. Was it a single compound, a class of compounds, or the synergistic effects of a mixture of compounds? These are some of the questions the toxicologists and other scientists in health related fields are trying to answer. We as research chemists do not know the answer, but hopefully we have demonstrated techniques of classifying organic contaminants into smaller groups for ease of identification, through extraction, fractionation, partitioning, and glass capillary gas chromatography. With such a separation scheme, it is conceivable that a similar scheme could be used by toxicologists to test many fractions of a mutagenic extract rather than unpartitioned concentrates to determine biologic effect.

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